A Direct 5 ms Column Performance Comparison for Active Semi-Volatile Analytes

Application Note

Environmental

Authors

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Abstract

Consistent column inertness performance is essential for obtaining reliable results for active analytes on an ongoing basis. Inertness, or conversely, activity testing for capillary GC columns has typically not been done with demanding probes to verify consistent inertness performance for each column shipped. Inert columns will show less tailing and better recovery at low levels for active analytes. In this example, the column inertness performance of premium 20 m × 0.18 mm × 0.18 µm 5 ms columns from Agilent and Vendor R are compared. The active analytes in the semi-volatile sample set illustrate the value of using columns with consistent, verified inertness performance.

The columns selected for this comparison were 20 m × 0.18 mm × 0.18 µm columns, because this format provides a means of conducting large numbers of semi-volatile analyses quickly. The 20 m × 0.18 mm × 0.18 µm format delivers the same resolution as the more popular 30 m × 0.25 mm × 0.25 µm column format, typically with a 30 to 40 percent improvement in analysis speed.

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Introduction

Semi-volatile analyses using methods similar to US EPA method 8270(1) are important in environmental laboratories worldwide. A number of very active analytes in this sample set present significant challenges for analysts, equipment providers and column manufacturers in terms of inertness. Acidic compounds such as benzoic acid or 2,4-dinitrophenol and strong bases such as pyridine or benzidine are examples of active species found in this sample set. These highly polar species are particularly susceptible to adsorption onto active surfaces in the sample flow path including the column itself. Both column and inlet inertness are critical for effective analysis of these active chemical species.

Inertness verification for the Agilent J&W Ultra Inert series of capillary GC columns is performed with aggressive active probes at low concentration and low temperature [2-4]. This is a rigorous approach that establishes consistent baseline inertness profiles for each column in the Agilent J&W Ultra Inert Capillary GC column series. The verified inertness profile then serves as a predictor for successful analysis of chemically active species that often tail and adsorb onto active sites on the column. These effects are particularly evident at trace levels with the active semi-volatile analytes in this application example.

In this application note, capillary GC columns from Agilent and Vendor R are compared using a subset of the most active semi-volatile analytes. The semi-volatile sample set includes a variety of analytes that tend to adsorb onto active sites throughout the GC flow path. Particularly difficult semi-volatile analytes include acidic species such as benzoic acid or 2, 4-dinitrophenol, and basic species such as aniline or benzidine. Liner or column activity, or both, can lead to poor peak shapes, including tailing, poor quantification, and in extreme cases complete disappearance of these analytes at low levels. Comparison of these columns using these active semi-volatile analytes illustrates just how critical consistent column inertness performance is to successful semi-volatile analyses.

The columns selected for this comparison were in a 20 m × 0.18 mm × 0.18 µm or high efficiency capillary GC column format. Established methods on 30 m × 0.25 mm × 0.25 µm columns can be translated easily to this smaller bore column format with Method Translation Software [5] while typically realizing a 30 to 40 percent savings in analysis time. More samples can be analyzed faster using a shorter 0.18 mm id high efficiency column with the same resolution. Time savings and the resulting productivity gains for high volume semi-volatiles analyses can lead to significant cost improvements in the highly competitive contract analytical laboratory marketplace.

Experimental

This work was done using an Agilent 6890N network GC system equipped with an Agilent 5975B Series MSD detector and an Agilent 7683B automatic liquid sampler. Details of the chromatographic conditions are presented in Table 1 below. Column comparisons were executed using the same GC, inlet liner, test solutions, and chromatographic conditions for each column evaluated. Each column in this study was installed as a new column, conditioned according to the manufacturers' instructions, and evaluated immediately without exposure to other samples or extraneous factors that could lead to column damage. The goal was to keep all factors constant with the exception of the column, in order to provide a valid comparison.

Table 1. Chromatographic Conditions

GC	Agilent 6890N network GC system
Sampler:	Agilent 7683B automatic liquid sampler, 5 µL syringe (Agilent part # 5181-1273), 0.5 µL injection
Inlet:	Splitless at 280 °C, septum purge flow 30 mL/min on at 0.75 min
Inlet Liner:	Deactivated dual taper direct connect (Agilent p/n 1544-80700), non stick O-ring (Agilent p/n 5188-5365), gold plated inlet seal (Agilent p/n 5188-5367)
Carrier:	Helium ramped flow 0.7 mL/min (0.1 min) to 1.3 mL/min at 15 mL/min^2 purified through ReNEWable trap (Agilent p/n G3440-60003)
Column 1:	20 m × 0.18 mm × 0.18 µm Agilent J&W HP-5ms Ultra Inert (Agilent p/n 19091S-577UI)
Column 2:	20 m × 0.18 mm × 0.18 µm Vendor R Rxi® 5 ms
Oven:	35 °C (0.1 min); 85 °C/min to 160 °C; 20 °C/min to 260 °C (0.20 min); 25 °C/min to 285 °C; 40 °C/min to 300 °C (3.5 min)
Detection:	MSD source 300 °C, quadrupole 180 °C, transfer line 290 °C, scanning mode 50-550 *m/z*

The flow path supplies used in these experiments are listed in Table 2 below.

Table 2 Flow Path Supplies

Vials:	Amber crimp cap vials (Agilent p/n 5182-0716)
Vial caps:	Red crimp caps (Agilent p/n 5282-0723)
Vial inserts:	100 µL glass/polymer feet (Agilent p/n 5181-1270)
Syringe:	5 µL (Agilent p/n 5181-1273)
Septum:	Advanced green (Agilent p/n 5183-4759)
Inlet liners:	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Ferrules:	0.4 mm id short; 85/15 Vespel/graphite (Agilent p/n 5181-3323)
	0.4 mm id long; 85/15 Vespel/graphite (Agilent p/n 5062-3508)
20x magnifier:	20x Magnifier loop (Agilent p/n 430-1020)

Sample Preparation

Semi-volatile short mix standard solutions (US EPA 8270) were obtained from Ultra Scientific, North Kingstown, RI 02852-USA. Burdick and Jackson Ultra RESI grade dichloromethane was purchased through VWR International, West Chester, PA 19380-USA. Solutions were prepared using dichloromethane solvent and class A volumetric pipettes and flasks.

Results and Discussion

The components of the semi-volatile short mix are a cross section of the most difficult analytes in the set. System performance can be evaluated quickly and effectively by examining the chromatographic behavior of these relatively few analytes. Early eluting nitrosamine and late eluting perylene bracket the time frame for the sample set. Any peak tailing of benzoic acid and 2, 4 dinitrophenol indicate column inertness issues with basicity, whereas peak tailing of aniline and benzidine indicate issues with acidity.

Peak symmetry is an important factor to consider when evaluating a chromatographic data set for semi-volatile analyses. Active analytes in the sample set can lead to tailing peak shapes, difficulty in integration, and potentially false negative results. This can occur if the GC flow path, or more importantly the GC column due to its large surface area, are not inert. These effects are often evident at lower concentrations. At higher concentrations, overloading of active sites which mask their impact, is more likely to occur. Figures 1 and 2 show total ion chromatograms (TIC) (Scan Mode) of a 0.5 nanogram on column loading for each of the analytes and internal standards in the short mix. At this low loading level, compound specific tailing or column activity is easy to recognize. The TIC in Figure 1 was produced on an Agilent J&W HP-5ms 20 m × 0.18 mm × 0.18 µm High Efficiency Capillary GC column while the TIC in Figure 2 was produced on a column from Vendor R in the same column format.

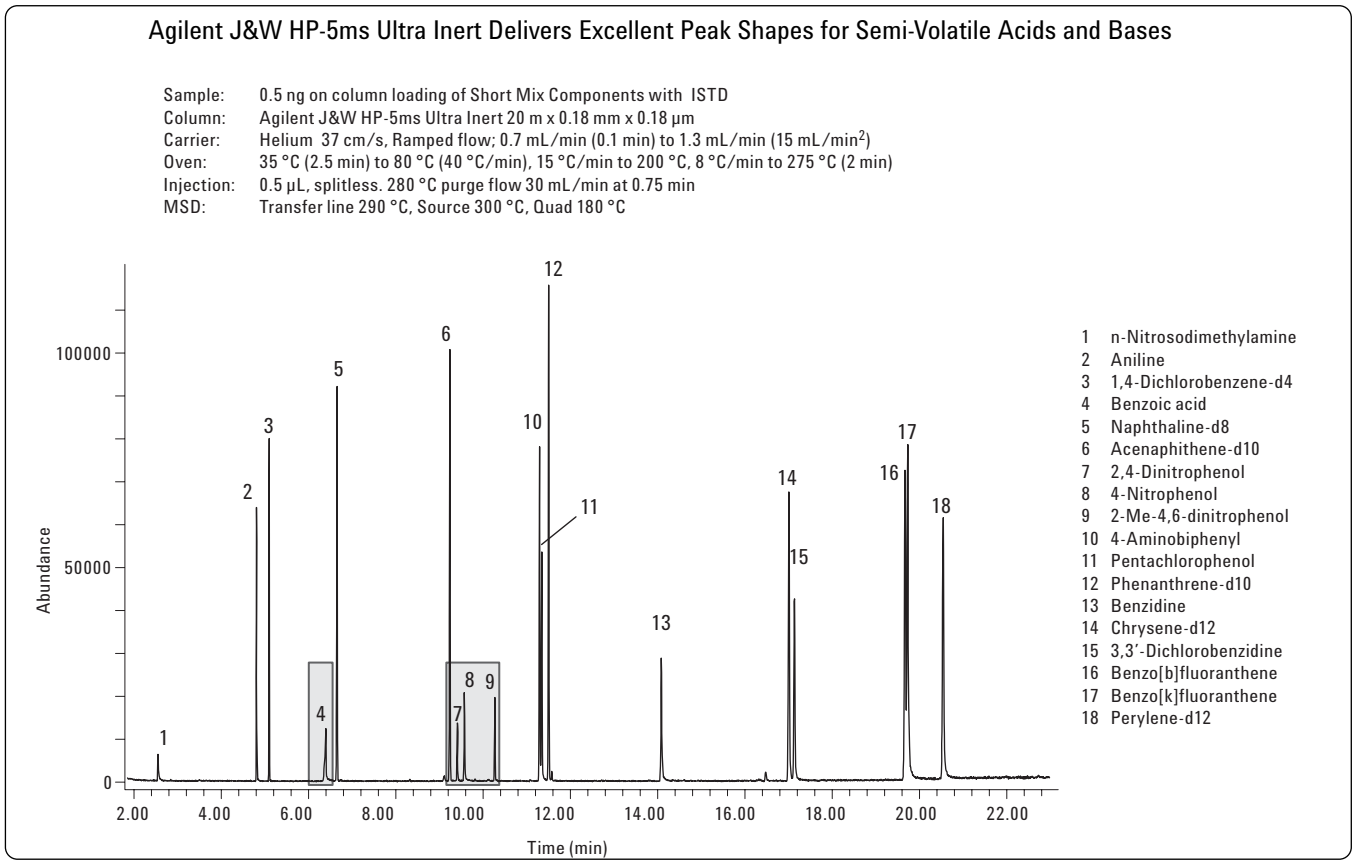

Figure 1. Total ion chromatogram of the EPA8270 short mix with a 0.5 nanogram on column loading on an Agilent J&W HP-5ms UI 20 m × 0.18 mm × 0.18 µm column. The acidic analytes in the sample are highlighted.

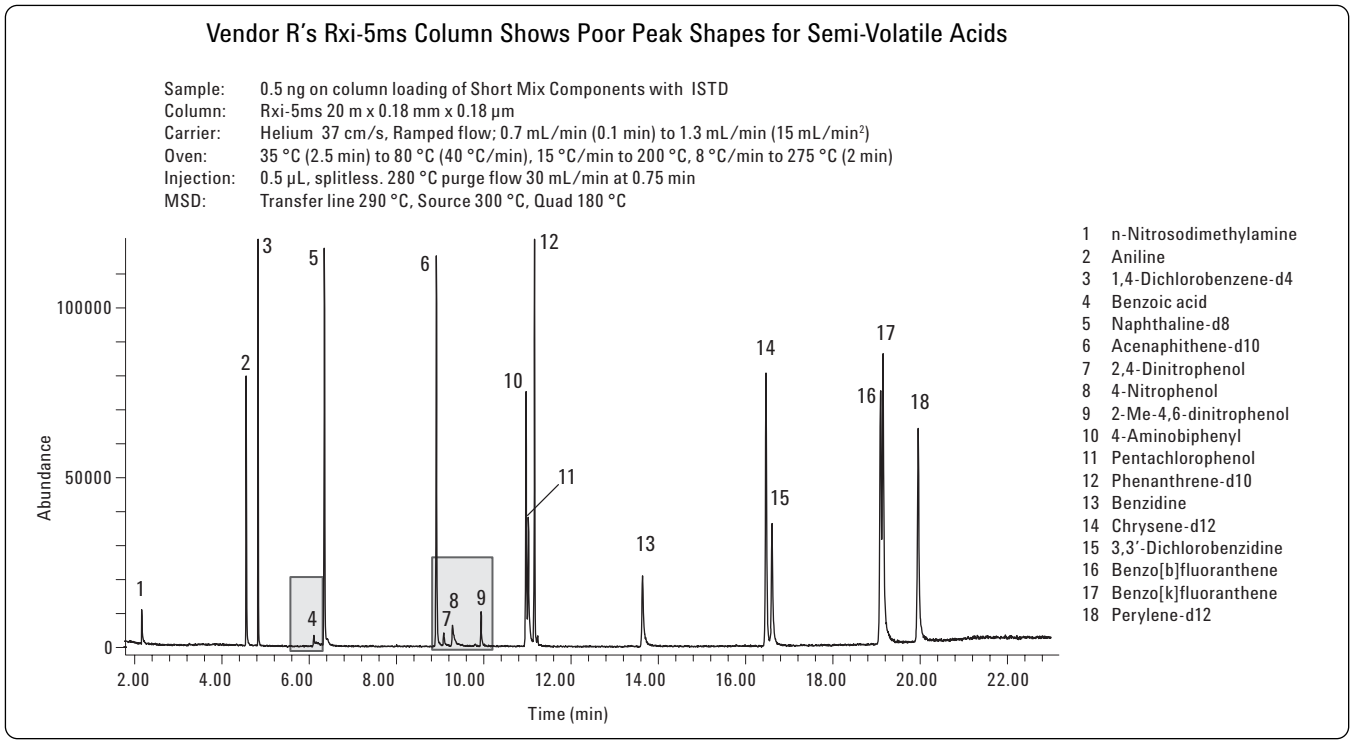

Figure 2 Total ion chromatogram of the EPA8270 short mix with a 0.5 nanogram on column loading on Vendor R's 20 m x 0.18 mm x 0.18 µm column. The acidic analytes in the sample are highlighted.

The relative response ratio (RRR) for 2,4-dinitrophenol (2,4 DNP) is a key acceptance criteria for establishing system suitability before initiating sample analysis. According to the method the relative response ratio of 2,4-dinitrophenol to the internal standard acenaphthene-d10 can be as low as 0.05 over the range of the analysis. In practice, low response factors are typically seen near the level of the lowest standard. Beginning a sample set with RRRs at or near the lower limit for the low end of the calibration curve specified is not a good idea in practice. The system is likely to drift rapidly out of suitability which in turn leads to costly re-analysis and lost billable sample hours. Summary data for the RRR results for 2,4-dinitrophenol is presented in Table 3 for each of the four columns evaluated in this comparison.

Relative response ratio data for 2,4-dinitrophenol on the Agilent J&W High Efficiency Capillary columns were higher across the range studied than Vendor R's columns. This effect was most evident at the low end on the calibration curve where the average RRR ratio values on the Agilent J&W columns were 0.136 and 0.158 while Vendors R columns' RRRs were 0.053 and 0.074. Having RRR values for 2,4 dinitrophenol near the 0.05 limit is a major concern before beginning the analysis of a large sample set. Starting a long sample set very close to the RRR limit will impact reportable detection limits for 2,4 dinitrophenol almost immediately and lead to costly reanalysis. Starting the same sample set with RRR values greater than 0.13 for the low level standard provides confidence that system suitability will continue longer and that lower reportable detection limits can be maintained.

Other acidic species in the EPA8270 short mix gave poor peak shape and lower recovery on Vendor R's columns when compared with the Agilent J&W HP-5ms Ultra Inert Capillary GC columns. The peak shape for peak 4, benzoic acid, in Figure 2 was tailing severely while the same peak at the same concentration in Figure 1 gave a sharp well-defined peak on the Agilent column. Better peak shapes and higher responses were also observed on the Agilent column in Figure 1 than Vendor R's column in Figure 2 for 4-nitrophenol and 2-Me-4,6-dinitrophenol. The peak shapes and recovery for each of the acidic species in the EPA8270 short mix was better on the Agilent column suggesting an issue with basicity on Vendor R's column.

Table 3. Relative Response Ratio Data for 2,4-Dinitrophenol on the Four Columns Tested in this Comparison

Relative Response Ratios (RRR) Data for 2,4-dinitrophenol (2,4 DNP)								
2,4 DNP µg/mL	Agilent J&W HP-5ms UI column 1	RRR avg. for level	Agilent J&W HP-5ms UI column 2	RRR avg. for level	Vendor R 5 ms column 3	RRR avg. for level	Vendor R 5 ms column 4	RRR avg. for level
1	0.136		0.164		0.044		0.073	
1	0.135		0.169		0.057		0.074	
1	0.136		0.162		0.051		0.078	
1	0.136	0.136	0.138	0.158	0.061	0.053	0.070	0.074
2	0.135		0.151		0.066		0.081	
2	0.134		0.156		0.060		0.078	
2	0.142		0.150		0.080		0.086	
2	0.143	0.138	0.147	0.151	0.081	0.072	0.085	0.083
5	0.161		0.165		0.095		0.116	
5	0.166		0.174		0.110		0.096	
5	0.178		0.173		0.115		0.125	
5	0.155	0.165	0.173	0.171	0.122	0.110	0.129	0.116
10	0.193		0.191		0.135		0.123	
10	0.200		0.205		0.159		0.166	
10	0.194		0.202		0.166		0.160	
10	0.207	0.199	0.204	0.201	0.162	0.155	0.177	0.156
20	0.219		0.224		0.192		0.198	
20	0.238		0.239		0.209		0.194	
20	0.248		0.243		0.245		0.187	
20	0.246	0.238	0.247	0.238	0.247	0.223	0.213	0.198
40	0.268		0.257		0.233		0.242	
40	0.274		0.270		0.246		0.239	
40	0.279		0.274		0.252		0.250	
40	0.281	0.275	0.277	0.270	0.261	0.248	0.258	0.247
Overall Average		0.192		0.198		0.144		0.146

Conclusions

Use of the short mix provided a quick and clear means of evaluating system performance for semi-volatile analysis. The challenging analytes used in this mix provided a framework to evaluate retention windows and the peak shapes of acid and base species. Maintaining sufficient resolution between peaks enabled close examination of chromatographic behaviors. Once the system equilibrated, the short mix gave an excellent view of system behavior with just a few injections.

For analysis of active analytes, consistent inertness performance from the column is essential for accurate results. Testing with deliberately active probes for column activity is the best means to verify that each column shipped will provide consistent inertness performance each time. Without this testing, consistency may decline as illustrated here with Vendor R column data. Relative response ratio values from the Vendor R column for 2,4 dinitrophenol were dramatically lower near the low end of the calibration range. The RRRs were also lower across the analysis range than the Agilent J&W HP-5ms Ultra Inert Capillary GC columns used in this comparison.

Inertness verification testing really does matter. Testing with truly active probes is the only reliable way of achieving consistent inertness performance with each column. Using columns with consistently low and verified levels of activity means better peak shapes and better quantification for active analytes. For consistent peak shapes and recovery of active analytes, this column comparison clearly shows that Agilent J&W HP-5ms Ultra Inert Capillary GC columns are the better choice.

Reference

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4. "Agilent J&W Ultra Inert GC Columns: A New Tool to Battle Challenging Active Analytes" Technical Overview, 5989-8685EN, May 29, 2008
5. Method Translation Software available by free download from the link below:

   www.agilent.com/chem/gcmethodtranslation

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